

A Simple and Sensitive LC-MS/MS Method for the Determination of Free 8-Hydroxy-2'-deoxyguanosine in Human Urine

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Abstract:

Urinary free 8-hydroxy-2'-deoxyguanosine (8OHdG), an oxidized product of DNA, and is frequently chosen as a biomarker of oxidative stress in humans, including studies of oxidative DNA damage during space flight. It is challenging to accurately and efficiently quantify urinary free 8OHdG in large scale human studies. LC-MS/MS is emerging as a preferable analytical technique owing its high sensitivity, selectivity and efficiency, compared to some traditional methods such as ELISA and HPLC.

A simple and sensitive LC-MS/MS method has been developed for the determination of free 8OHdG in human urine. Sample preparation was done by solid phase extraction with a Waters Oasis HLB 96 well plate. A Waters Alliance 2795 HT Separation Module combined with a Quattro Micro tandem mass spectrometer was used as the LC-MS/MS system. The runtime of one injection can be less than 5 minutes using a reversed phase C18 column and an isocratic flow of methanol/water. ESI positive ions were quantified in the multiple reaction modes (MRM) using m/z 284→168 for 8OHdG and m/z 289→173 for stable isotope labeled internal standard [¹⁵N₅] 8OHdG.

With this method for 8OHdG, a lower limit of quantitation of 1.0 nM (0.28 ng/mL) has been achieved using 100 μ L urine sample. The analytical range is between 1.0 and 100 nM with a correlation coefficient ≥ 0.99 . Good reproducibility can be obtained with intra-assay and inter-assay CVs $\leq 10\%$ for 8OHdG spiked urine QC samples. This method can be used in high-throughput routine analysis of free 8OHdG in human urine.